

AMENDMENT

Please amend the application as follows:

In the specification:

Please replace the title of the invention found on the application cover sheet and page 1, lines 1-2 with the following:

*Changed
from
Hahn* ~~NUCLEIC ACIDS FOR THE DIAGNOSIS AND TREATMENT OF GIANT CELL~~
ARTERITIS

Please replace the paragraph beginning at page 1, line 11 with the following rewritten paragraph:

D1 ~~¶~~ Giant cell arteritis (GCA) is a systemic vasculitis that is a serious and potentially blinding rheumatologic disease of the elderly. Current treatment of GCA requires systemic immunosuppression with profound morbidity in the affected elderly population. GCA is widely believed to be immune-mediated; however, the etiology and pathogenesis of this systemic vasculitis remains unidentified. Furthermore, diagnosis of GCA is difficult because it relies on a constellation of nonspecific signs and symptoms and a diagnostic arterial biopsy. Significantly, blindness may be the first symptom of GCA. Thus, if a way were found to better diagnose or even screen for early onset or predisposition for GCA at an earlier stage of the disease, many cases of blindness and many lives would be saved. ~~¶~~

Please replace the paragraph beginning at page 1, line 20 with the following rewritten paragraph:

D2 ~~¶~~ Currently, corticosteroids are critical in the treatment of giant cell arteritis; they reduce the incidence of blindness and rapidly relieve symptoms. However, the amounts of steroids (e.g., prednisone) needed are significant and not without side effects, particularly as they usually must be given over an extended period of time, usually about two years. Steroid treatment is not uniformly effective and causes significant morbidity in up to 40% of patients because of hypertension, osteoporosis, infection, glucose dysregulation, fluid overload, and aseptic necrosis

D2 of the hip or shoulder. Alternative use of nonsteroidal anti-inflammatory drugs (NSAIDs) will lessen the painful symptoms, but they do not prevent the blindness or vascular problems. Accordingly, new methods of treating GCA are needed. The present invention addresses these and other needs.

Please replace the paragraph beginning at page 4, line 25 with the following rewritten paragraph:

D3 ¶ The invention also provides kits, e.g., ELISA kits, for detecting the presence of human antibodies associated with GCA in a sample comprising a polypeptide of the invention. The polypeptides or peptides in the kit can be immobilized. The kit can further comprise a non-human antibody or antisera that specifically binds to a human antibody under *in situ* or *in vitro* conditions. As described below, the non-human antibody in the kit can further comprise a detectable tag (e.g., an enzyme, a radionuclide, biotin, and the like, as discussed below), or the invention can comprise a second antibody capable of binding to the first non-human antibody.

Please replace the paragraph beginning at page 5, line 5 with the following rewritten paragraph:

D4 ¶ The invention provides methods for diagnosing or determining predisposition for GCA comprising the following steps: (a) providing an antibody that specifically binds to a polypeptide associated with GCA, wherein the antibody has the same specificity as an antibody of the invention (that binds to a GCA-associated peptide or polypeptide); or, a nucleic acid that can detectably hybridizes to a nucleic acid of the invention under *in situ* or *in vitro* conditions; (b) providing a tissue or fluid (e.g., whole blood, serum or urine) sample; (c) contacting the antibody or nucleic acid with the sample; and (d) detecting whether the antibody specifically binds to a polypeptide in the tissue or serum sample or the nucleic acid hybridizes to a nucleic acid in the tissue or serum sample; wherein the specific binding or hybridization is diagnostic for or determines a predisposition for GCA.

Please replace the paragraph beginning at page 5, line 30 with the following rewritten paragraph:

D5 ¶ The invention provides methods for isolating nucleic acid sequences associated with GCA comprising the following steps: (a) providing a first tissue sample from a tissue or fluid specimen not showing histologic or other signs of GCA and a second tissue sample from a tissue or fluid specimen showing histologic or other signs of GCA; (b) isolating the nucleic acid from both samples; (c) subtracting nucleic acid from the first sample from the second sample to isolate nucleic acid only present in the second sample, wherein the isolated nucleic acid from the second sample is associated with GCA-affected tissue and not normal tissue. This aspect of the invention can incorporate all variations and equivalents of subtractive hybridization techniques, as described below. In this method, the first and the second tissue sections can be taken from a "skip" lesion of a temporal artery of a GCA patient. ¶

Please replace the paragraph beginning at page 8, line 10 with the following rewritten paragraph:

D6 ¶ The invention is based on the discovery that novel sequences can be associated with GCA lesions. While the invention is not limited by any particular theory or mechanism, these unique-GCA associated sequences may be associated with a pathology initiating or causative microorganism. Accordingly, subtractive hybridization of normal (non-involved) from GCA-involved tissue led to the discovery of the novel GCA-associated sequences of the invention. Translation of exemplary sequences to recombinant polypeptides (in the form of fusion proteins for convenience of isolation and manipulation) led to the discovery that GCA patients have circulating antibodies that specifically bind to the polypeptides of the invention. Accordingly, the peptides and polypeptides of the invention are used in kits and methods for diagnosing GCA by identifying circulating anti-GCA antibodies in the serum, urine or tissue samples of patients. Because blindness may be the first presenting symptom of GCA, the diagnostic methods of the invention can be used to screen for GCA on patients that, while having no symptoms of GCA, do have a relatively high probability of suffering from GCA, such as elderly patients. ¶

Please replace the paragraph beginning at page 19, line 15 with the following rewritten paragraph:

D7
One exemplary means to biopsy, or isolate, GCA lesions is by dissection with laser-capture microdissection (LCM), either freshly biopsied or archival pathology specimens of GCA-positive arteries from both histopathologically involved and uninvolved areas. Because the vasculitis of GCA occurs in an irregular, or discontinuous pattern (the so-called "skip lesion"), isolation of one artery (or vein, if appropriate) sample can yield both involved and uninvolved tissue samples. Retrieval of selected cells is achieved by activation of a transfer film placed in contact with a tissue section, by a laser beam (30 or 60 micron diameter) that is focused on a selected area of tissue using an inverted microscope. In LCM, a thermoplastic polymer coating (e.g., ethylene vinyl acetate) attached to a rigid support is placed in contact with a tissue section. The EVA polymer over microscopically selected cell clusters is precisely activated by a near-infrared laser pulse and bonds to the targeted area. Removal of the EVA and its support from the tissue section procures the selected cell aggregates for molecular analysis. A computer-controlled arm can precisely position a 40-micron-wide strip of a cylindrical EVA surface onto a sample with a light contact force. Techniques of laser-capture microdissection are known in the art, e.g., the PixCell laser capture microdissection (LCM) system, see, e.g., Kohda (2000) Kidney Int. 57:321-331; Goldsworthy (1999) Mol. Carcinog. 25:86-91; Banks (1999) Electrophoresis 20:689-700; Emmert-Buck (1996) Science 274:998-1001; U.S. Patent Nos. 5,985,085; 5,859,699.

Please replace the paragraph beginning at page 20, line 5 with the following rewritten paragraph:

D8
Nucleic acids within the scope of the invention include isolated or recombinant nucleic acids which specifically hybridize to an exemplary nucleic acid of the invention. Stringent conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen, Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Probes, "Overview of principles of hybridization and the strategy of

D8 nucleic acid assays" (1993). Generally, stringent conditions are selected to be about 5 to 10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50% of the probes are occupied at equilibrium). Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide.*

Please replace the paragraph beginning at page 27, line 3 with the following rewritten paragraph:

D9 *Another example of an algorithm that is suitable for determining percent sequence identity (*i.e.*, substantial similarity or identity) in this invention is the BLAST algorithm, which is described in Altschul (1990) *J. Mol. Biol.* 215:403-410. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul (1990) *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues, always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation

of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. In one embodiment, to determine if a nucleic acid sequence is within the scope of the invention, the BLASTN program (for nucleotide sequences) is used incorporating as defaults a wordlength (W) of 11, an expectation (E) of 10, M=5, N=4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as default parameters a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see, *e.g.*, Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).

Please replace the paragraph beginning at page 66, line 7 with the following rewritten paragraph:

In various embodiments of the invention, the polypeptides (or peptides) and antibodies of the invention are immobilized to the "capture" GCA-associated antibodies or polypeptides, respectively. Additional reagents are added to this reaction to detect any specific binding. These so-called "sandwich assays" are commercially useful for detecting or isolating protein or antibodies.

Please replace the paragraph beginning at page 67, line 14 with the following rewritten paragraph:

Immunoassays can be either competitive or noncompetitive. Noncompetitive immunoassays are assays in which the amount of captured analyte (GCA-associated human antibody) is directly measured. In one "sandwich" assay, for example, the capture agent (a polypeptide or peptide of the invention) can be bound directly to a solid substrate where they are immobilized. These immobilized reagents then capture antibody present in the test sample. The antibody thus immobilized is then bound by a labeling agent, such as a second anti-human antibody reagent bearing a label. Alternatively, the human antibody binding reagent can lack a label, but it may, in turn, be bound by a labeled third reagent (*e.g.* another antibody), *e.g.*, specific to antibodies of the species from which the second antibody is derived. The second (or third) can be modified with a detectable moiety, such as biotin, to which another labeled molecule can specifically bind, such as, *e.g.*, enzyme-labeled streptavidin. In a variation of the above, the immobilized reagent can be an antibody of the invention used to capture a GCA-

D11 associated polypeptide. The second (soluble) reagent can be, e.g., another GCA-associated polypeptide binding antibody of the invention. Competitive binding assays can also be used. For example, a known amount of labeled human antibody is added to the serum or tissue sample. The sample is then contacted with the capture agent (GCA-associated polypeptides or peptides of the invention). The amount of labeled human antibody bound to the immobilized reagent is inversely proportional to the concentration of GCA-polypeptide reactive antibody present in the sample. A hapten inhibition assay is another competitive assay.

Please replace the paragraph beginning at page 74, line 18 with the following rewritten paragraph:

D12 ¶Pharmaceutically acceptable carriers can contain a physiologically acceptable compound that acts, e.g., to stabilize the composition or to increase or decrease the absorption of the agent and/or pharmaceutical composition. Physiologically acceptable compounds can include, for example, carbohydrates, such as glucose, sucrose, or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins, compositions that reduce the clearance or hydrolysis of any co-administered agents, or excipients or other stabilizers and/or buffers. Detergents can also be used to stabilize the composition or to increase or decrease the absorption of the pharmaceutical composition (see infra for exemplary detergents).

Please replace the paragraph beginning at page 76, line 24 with the following rewritten paragraph:

D13 ¶The pharmaceutical compositions of the invention (e.g., therapeutic antibodies, vectors or antisense oligonucleotides) can be delivered by any means known in the art systemically (e.g., intravenously), regionally, or locally (e.g., intra- or peri-tumoral or intracystic injection, e.g., to treat bladder cancer) by, e.g., intraarterial, intratumoral, intravenous (IV), parenteral, intra-pleural cavity, topical, oral, or local administration, as subcutaneous, intra-tracheal (e.g., by aerosol) or transmucosal (e.g., buccal, bladder, vaginal, uterine, rectal, nasal mucosa), intra-tumoral (e.g., transdermal application or local injection). For example, intra-arterial injections can be used to have a "regional effect," e.g., to focus on a specific organ (e.g., brain, liver,

D13 spleen, lungs), for example, intra-hepatic artery injection or intra-carotid artery injection. If it is desired to deliver the preparation to the brain, it can be injected into a carotid artery or an artery of the carotid system of arteries (e.g., occipital artery, auricular artery, temporal artery, cerebral artery, maxillary artery, etc.).

Please replace the paragraph beginning at page 79, line 15 with the following rewritten paragraph:

D14 Thirty sequentially cut tissue sections (10 μ m each) were placed on non-charged microscope slides, fixed, dehydrated, and stained with hematoxylin and eosin. GCA inflammatory lesions were identified and dissected using a Laser Capture Microdissecting Microscope™ (Arcturus Engineering, Inc., Mountain View, CA). Approximately 500 cells including giant cells and inflammatory infiltrate were dissected from each GCA specimen. DNA isolated from these cells represented the "tester" population. Approximately 500 cells were microdissected from a subsequent section with no histopathologic evidence of GCA lesions. The DNA isolated from these cells represented the "driver" sample in the RDA.

Please replace the paragraph beginning at page 80, line 12 with the following rewritten paragraph:

D15 One criterion for selecting which genes would first be translated into recombinant polypeptides and used to analyze for the presence of human antibodies in serum from GCA patients was relative homology to sequences of known microbial origin. A total of eleven unique DNA sequences were obtained in the first two RDAs using two different GCA+ arterial specimens. Sequence identity analysis showed that four of the unique sequences, GCA 1, GCA 5, GCA 14, and GCA 17, may be distantly related to known microbial sequences. Thus, these were the first sequences selected for further characterization.

Please replace the paragraph beginning at page 83, line 4 with the following rewritten paragraph:

D16 Human sera was tested in triplicate at multiple dilutions in 0.05% Tween20 -PBS. Reactivity was detected with an alkaline-phosphatase-labeled goat anti-human IgG and